Expression of uterine oxytocin receptors and blood þÿ p r o g e s t e r o n e , 1 3 , 1 4 - d i h y d r o - 1 5 - k e t o - p r o s t a g l a n ionized calcium levels in dystocic bitches

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EXPRESSION OF UTERINE OXYTOCIN RECEPTORS AND BLOOD PROGESTERONE, 13,14-DIHYDRO-15-KETO-PROSTAGLANDIN F_{2A}, AND IONIZED CALCIUM LEVELS IN DYSTOCIC BITCHES

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Abstract

This study aimed to examine the etiology of canine dystocia by measuring the relative expression of oxytocin receptor (OXTR) mRNA and the concentration of serum progesterone, plasma $PGF_{2\alpha}$ metabolite (PGFM), and blood ionized calcium (iCa) near term and in dystocia. Altogether 58 bitches were included in this study, 41 of which underwent cesarean section (CS). The four CS groups were based on history: complete uterine inertia (CUI; n = 7), partial uterine inertia (PUI; n = 13), obstructive dystocia (OD; n = 10), and elective cesarean section (ECS; n = 11). An additional group of medically treated dystocia without CS (MD; n = 8) and a control group (C; n = 9) with normal parturition (without CS and medical treatment) were also formed. Blood samples were taken prior to CS or medical treatment. Progesterone concentrations were highest in the ECS and a significant difference ($p<0.05$) was observed between the ECS and the OD and between the ECS and the combined dystocia (CUI, PUI, OD, MD) groups (COMB). Highest concentrations of PGFM was observed in the C, the difference being significant ($p<0.05$) between the C and the ECS and between the C and the COMB group. The progesterone:PGFM ratio was significantly ($p<0.05$) higher in the ECS than in the C and the COMB group. No significant difference ($p>0.05$) was observed in iCa concentrations between the groups. Relative OXTR mRNA expression was evaluated with real-time PCR from full-thickness uterine samples taken from the incision site during CS. The expression was highest in the ECS and the difference in expression was significant ($p<0.05$) between the ECS and the OD and between ECS and the combined dystocia (CUI, PUI, OD) groups (COMB2). The study supports previous reports of decreasing progesterone and increasing PGFM during prepartum luteolysis. Upregulation of OXTR occurs near term. In obstructive dystocia, a prolonged influence of oxytocin and uterine exhaustion may lead to downregulation of OXTR. Complete primary uterine inertia may have a different etiology as no clear decrease in OXTR was observed in CUI as in OD. It

remains unclear if parturition ceases because of uterine inertia or if uterine inertia occurs because of ceased parturition and desensitization of receptors.

Keywords:

Canine, uterus, birth, progesterone, prostaglandin $F_{2\alpha}$

1. Introduction

Parturition is a complex event and includes hormonal and behavioral changes, neural activity, and interaction between the dam and the offspring. Near term, canine plasma $PGF_{2\alpha}$ levels increase leading to luteolysis, followed by a decrease in peripheral plasma progesterone levels [1,2,3] that allow for contractions of the uterus and for parturition to proceed [1]. The secretion of $PGF_{2\alpha}$ in the bitch is suggested to originate from placental trophoblast cells [4]. During parturition, increase in peripheral plasma cortisol [2,5,6], vasopressin [6], and oxytocin (OT) [6,7] occur. However, changes in cortisol levels vary greatly between individuals during parturition [2,5,6]. While estrogen concentrations are somewhat higher in the last trimester of pregnancy in the bitch, there is no marked prepartum increase as detected in many other species [8,9,10]. Two days prior to parturition, estrogen levels of the bitch decrease suddenly during prepartum luteolysis indicating its luteal source [11].

Occurrence of dystocia in bitches varies greatly depending on the population studied; the average is estimated to be below 5% [12]. In a group of 200 000 insured bitches (excluding Boston Terrier, English Bulldog, and French Bulldog) in Sweden, dystocia occurred in 16% of parturitions [13]. In the UK, the occurrence varied from 0% to 92% among 151 breeds (22, 005 litters) [14]. While

dystocia seems to be more common in miniature and small breeds [13,15,16], several medium- and large-size breeds also have a higher than average proportion of litters born by cesarean section (CS) [14]. Approximately 60% of dystocia cases undergo CS [13,15,16,17]. In brachycephalic breeds, the proportion of CS is very high [14,18]. There may be a risk of bias in statistics of dystocia in these breeds due to the popularity of elective CS (ECS).

Dystocia is sometimes difficult to diagnose. Therefore, a complete history and physical examination is required. The suggested causes vary slightly according to different authors [12,19,20]. There are several, sometimes simultaneous, causes of dystocia. Maternal factors are more common than fetal factors. The most common maternal cause is primary uterine inertia, which can be complete or partial [12]. In complete primary uterine inertia, the uterus fails to initiate parturition due to absence of uterine contractions and thus no puppies are born [12,17]. In partial primary uterine inertia, the bitch may have weak uterine contractions or contractions that cease without any obvious reason (such as obstruction) before all puppies are born [12,17]. Secondary uterine inertia is caused by prolonged parturition due to obstruction in the birth canal [12,17].

Oxytocin is a nonapeptide hormone produced mainly in the hypothalamus and stored in the posterior pituitary gland. Oxytocin is released after suitable stimulus, such as intracervical pressure. As one of the most potent uterotonic hormones, OT enhances the contractility of the uterus. During parturition, plasma OT concentration increases [6,7]; this may not occur in dystocia [21,22]. The effect of OT in the uterus is mediated through specific, class I G-protein-coupled transmembrane receptors known as oxytocin receptors (OXTR) [23]. Near term, during prepartum luteolysis, OXTR are upregulated [24,25,26]. In humans, continuous exposure to OT leads to desensitization of OXTR by reduction of OT binding sites in the myometrial cell membrane and by downregulation of OXTR mRNA in myometrial cells [27]. While desensitization may also have a role in canine dystocia due to prolonged

91 influence of OT, there is no published evidence of OXTR desensitization in bitches. The aim of this
92 study was to examine the relative expression of OXTR mRNA in the canine uterus near parturition
93 and in dystocia. Levels of serum progesterone, plasma prostaglandin $F2_{\alpha}$ metabolite (PGFM),
94 progesterone:PGFM ratio, and blood ionized calcium (iCa) were also analyzed to clarify possible
95 causative factors for dystocia.
96
97
98 **2. Materials and methods**
99
100 The study was approved by the Ethics Committee of the Viikki Campus, University of Helsinki,
101 Finland. Blood sampling from bitches with normal parturition was authorized by the National Animal
102 Experiment Board (ESAVI, Hämeenlinna, Finland), license number ESAVI/3802/04.10.03/2011.
103
104 *2.1. Groups*
105
106 Client-owned pet bitches that had CS performed either at the Small Animal Clinic of Mäntsälä or the
107 Veterinary Teaching Hospital of the University of Helsinki were enrolled in the study (Table 1). The
108 inclusion criteria were a diagnosis of dystocia resulting in CS or ECS due to small litter size or
109 previous dystocia. In addition, one group was established from bitches with medically treated mild
110 dystocia that gave birth without CS. Bitches with normal parturitions served as controls for blood
111 parameters. The owners of the bitches were requested to sign a written consent and complete a
112 questionnaire to obtain the history of the bitch including previous and present parturitions. Any
113 systemic disease was an exclusion criterion.
114

115 The following study groups were formed: 1) complete primary uterine inertia (CUI; n = 7, no puppies
116 born, parturition does not proceed, discharge of fetal fluids >3 hours or green discharge, no response
117 to vaginal stimulus), 2) partial primary uterine inertia (PUI; n = 13, at least one puppy born, parturition
118 ceases without obstruction), 3) obstructive dystocia (OD; n = 10, fetal oversize/narrow birth canal,
119 malpresentation, malformation), 4) elective caesarean section (ECS; n = 11, 58-66 days from mating,
120 previous dystocia, one or two puppies, before the onset of the stage 1 of parturition), 5) medically
121 treated dystocia (MD; n = 8, no CS, medical treatment), 6) control (C; n = 9, no CS, no medical
122 treatment, normal parturition). Dystocia groups were also combined (COMB: CUI, PUI, OD, MD
123 and COMB2: CUI, PUI, OD) to compare with ECS and C. The diagnosis and treatment decisions
124 were performed by the veterinarian on call. After blood sampling, the bitches were treated, if
125 necessary, with calcium glubionate (Calcium-Sandoz®, Sandoz A/S, Copenhagen, Denmark) and
126 oxytocin (Vetox®, Vetcare, Salo, Finland) (Table 1).
127
128 The individual and average data of the bitches are presented in Table 1. Altogether 35 different breeds
129 were included in the study. Four bitches in the ECS group had had previous history of dystocia and
130 CS. In the PUI and MD groups, each had one bitch with mild, medically treated dystocia without CS
131 in the previous pregnancy. The previous parturitions of the other multiparous bitches were normal.
132 The gestation length was calculated from ovulation day (at progesterone level 16-32 nmol/l) and from
133 the first and the last mating according the available information (Table 1).
134
135 *2.2. Blood sampling*
136
137 Blood samples were taken prior to CS or medical treatment from the vena cephalica into a syringe
138 (Radiometer Safe Pico, ref: 956-610, Radiometer Medical, Copenhagen, Denmark), to an EDTA tube
139 (Vacuette®, Mekalasi Oy, Nurmijärvi, Finland) with 5000 KIU aprotinin/ml EDTA blood (Aprotinin,

Roche Diagnostics GmbH, Mannheim, Germany) and a serum tube with clotting activator (Vacuette®, Mekalasi Oy, Nurmijärvi, Finland). Blood samples were taken prepartum in the ECS group and peripartum (second stage of parturition) in the other groups. EDTA tubes and syringes were stored in an ice-water bath and serum tubes at room temperature. Blood samples were centrifuged (Eppendorf Centrifuge 5810R, Eppendorf Nordic A/S, Hørsholm, Denmark) as follows: EDTA tubes at 4 °C, 1200 x *g*, 10 min, and serum tubes at 22 °C, 1700 x *g*, 10 min. Plasma and serum were divided into aliquots, frozen at -20 °C, and stored at -70 °C until analyzed.

2.3. Progesterone assay

Serum progesterone concentrations were measured in one run using a commercial RIA kit (Progesterone Coat-A-Count® RIA, Siemens Healthcare Diagnostics Oy, Espoo, Finland) according to the manufacturer's instructions. The concentrations were measured in duplicate with a gamma counter (1272 GliniGamma, LKB Wallac Oy, Turku, Finland). The intra-assay coefficient of variation was 3.4% at a serum concentration of 4.4 nmol/L and 2.0% at a concentration of 32.5 nmol/L.

2.4. $PGF_{2\alpha}$ metabolite assay

Concentrations of the major metabolite of PGF2α, 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ (PGFM), were measured from plasma using a commercial immunoassay kit (DetectX® 13,14-dihydro-15-keto-$PGF_{2\alpha}$ (PGFM) Enzyme Immunoassay Kit, Arbor Assays, Michigan, USA) according to the manufacturer's instructions. Prior to performing the assay, plasma samples were diluted 1:15 with the assay buffer provided in the kit. The optical density of each well was measured with a Multiscan GO Spectrophotometer with SkanIt software 4.1 (Thermo Fisher Scientific Oy, Vantaa, Finland).

The intra-assay coefficient of variation of duplicates was 12.0%. The inter-assay coefficient of variation was 13.0% at a plasma concentration of 15.8 nmol/L and 3.5% at a concentration of 58.5 nmol/L. The linearity of the assay was evaluated by diluting the canine plasma sample (1/10, 1/20 and 1/40) with the assay buffer provided in the kit. Observed to expected ratios were calculated for the dilutions. The mean recovery of the expected PGFM concentrations in different dilutions was 102% and dilutions of the canine plasma sample showed linearity over the studied range (R^2=0.996). The detection limit was 0.13 nmol/L.

2.5. iCa

Blood iCa was analyzed instantly with Roche Electrolyte Analyzer (9180, Fisher Scientific Oy, Vantaa, Finland) from Safe Pico syringes stored in an ice-water bath. The syringes contained 60 IU of dry electrolyte-balanced heparin. Contact with air was minimized with a specific cap to remove possible air bubbles.

2.6. Uterine samples

Uterine samples were obtained only from bitches undergoing a CS. Immediately after the removal of the puppies from the uterus, a full-thickness sample of uterine wall (approximately 5 x 30 mm) was taken from the incision site (interplacental area, uterine body or proximal horn). The sample was immediately frozen in liquid nitrogen and stored at -70 °C for PCR analysis to measure the relative expression of OXTR mRNA.

2.7. PCR

190 *2.7.1. RNA preparation and reverse transcription*

191 The full procedure has been described previously [28]. In brief, a 2-μg aliquot of total RNA from
192 each canine uterine sample was reverse transcribed at 37°C for 60 min in a final volume of 20 μL
193 with a reaction mixture (Qiagen) containing 1× RT buffer, dNTP mix (0.5 mM each dNTP), 600 ng
194 random primers (Invitrogen, Paisley, UK), 2 units of RNase inhibitor (Qiagen), and 4 units of
195 Omniscript™ reverse transcriptase (Qiagen).

196

197 *2.7.2. Real-time PCR analysis*

198 The real-time PCR analysis and the primers used have been described previously [28]. The
199 oligonucleotide primer pair for the OXTR was designed with NCBI/Primer-BLAST. To standardize
200 the quantification method, RPL27 and HPRT1 were selected as non-regulated reference genes with
201 primer pairs obtained from Silva et al. [29] and Bhatti et al. [30], respectively. The primers were
202 based on the sequences of the canine genes, and were the following: OTR forward primer: 5'-
203 TGCTGGCCTTCATCGTGTGCT-3'; OTR reverse primer: 5'-
204 GATGAAAGCCGAGGCTTCCTTGGG-3' from NM_001198659.1 with predicted size 95 bp;
205 RPL27 forward primer: 5'-ACAATCACCTCATGCCCACA-3'; RPL27 reverse primer: 5'-
206 CTTGACCTTGGCCTCTCGTC-3' from NM_001003102.2 with the predicted size 122 bp; HPRT1
207 forward primer: 5'-AGCTTGCTGGTGAAAAGGAC-3'; HPRT1 reverse primer: 5'-
208 TTATAGTCAAGGGCATATCC-3' from NM_001003357.1 with predicted size 104 bp. All samples
209 were run in duplicate and the purity of PCR products was confirmed by a melting-curve analysis in
210 all experiments. Each PCR assay included a negative control containing an RNA sample without
211 reverse transcription. The PCR amplification rate and the cycle threshold (C_t) values were analyzed
212 using iCycler™ iQ 3.1 software (Bio-Rad). The OXTR product was normalized against the mean of
213 RPL27 and HPRT1 products to yield the relative expression of OXTR mRNA.

2.8. Statistical analysis

Data were analyzed using IBM SPSS Statistics 24 software for Windows. The non-parametric Kruskal-Wallis one-way ANOVA test with Bonferroni correction was used to detect possible differences in serum progesterone levels, plasma PGFM levels, blood iCa, and relative expression of OXTR mRNA between the groups. Differences were considered statistically significant at $p<0.05$.

3. Results

3.1. Progesterone

Serum progesterone concentrations in the different groups are presented in Fig. 1a. The concentrations were highest in the ECS group; the largest variation in levels was also observed in this group. There was a significant difference ($p<0.05$) between the ECS and the OD and between the ECS and the COMB groups.

3.2. PGFM

Plasma PGFM concentrations were highest in the C and lowest in the ECS group (Fig. 1b). A significant difference ($p<0.05$) was detected between the C and the ECS and between the C and the COMB groups.

3.3. Progesterone:PGFM

The progesterone:PGFM ratio was highest in the ECS group (Fig. 1c). A significant difference ($p<0.05$) was observed between the ECS and the C and between the ECS and the COMB groups.

3.4. iCa

Blood iCa concentrations were lowest in the PUI group but no significant difference ($p>0.05$) was observed between the groups (Fig. 1d). No hypocalcemia was detected (reference interval 1.16-1.40 nmol/l).

3.5. qPCR

The mean relative expression of OXTR mRNA was highest in the ECS group (Fig. 1e). The difference was significant ($p<0.05$) between the ECS and the OD and between the ECS and the COMB2 groups. There was no significant difference between bitches treated or not treated with calcium glubionate and OT.

4. Discussion

Our study indicates that in complete primary uterine inertia the etiology may not be the absence or downregulation of OXTR, as there was no difference in OXTR expression in comparison of CUI to bitches near term but before the first stage of parturition (ECS group). Upregulation of OXTR occurs

near term, and the prolonged influence of OT and uterine exhaustion in obstructive dystocia may lead to downregulation of OXTR.

Our results support previous reports [1,2,3] on decreasing progesterone and increasing PGFM levels during prepartum luteolysis in pregnant bitches. As expected, progesterone levels were higher in the ECS group than in the other groups, as CS was performed in this group before the onset of parturition (before stage 1). A sudden decrease of progesterone is observed in near term pregnant bitches at the end of the luteal phase [11]. Termination of corpora lutea function in non-pregnant bitches is suggested to be more likely regressive than the active luteolytic process found in pregnant bitches, which indicates a different regulation mechanism [31]. Failure of luteolysis can lead to prolonged gestation [32]. Except in the ECS group, all bitches in this study had undergone luteolysis.

One possibility for the etiology of complete primary uterine inertia could be a problem in parturition initiation. Excessive progesterone and insufficient PGF2α levels could prevent sufficient uterine contractions and thus interfere with parturition. However, our results suggest that this might not be the case, as the progesterone and PGFM levels in CUI group were similar to other dystocia groups. This may indicate that the etiology is more likely at the level of uterine function, such as myometrial distention beyond its capacity to contract or the lack of cervical pressure to stimulate OT release. The progesterone:PGFM ratio was highest in the ECS group, where the highest progesterone and lowest PGFM concentrations were also found. This indicates that luteolysis had not yet occurred in this group. A high progesterone:PGFM ratio has been reported in dystocic bitches with complete primary uterine inertia in comparison to a control group [22]. In our study no such difference was observed.

Calcium and OT injections are used as a treatment for uterine inertia to enhance contractions of the uterus [19,20]. Batra [33] reported that OT-induced myometrial contractions in the rat depend on the

287 influx of extracellular calcium, and this influx is directly increased by OT. The action of OT has also
288 been postulated to occur by inhibiting the Ca^{2+}-extrusion pump in humans [34]. Hypocalcemia was
289 not diagnosed in any of the bitches in this study. However, there are reports of hypocalcemia in risk
290 groups of uterine inertia [35] and in dystocic bitches [36]. In our study, a single treatment with
291 calcium glubionate and OT did not seem to affect the expression of OXTR mRNA or distribution of
292 OXTR. In dystocia, the uterus has been under the influence of OT, and exhaustion and desensitization
293 may prevent medical treatment to induce uterine contractions. However, in this study the number of
294 bitches treated or not treated was low and further investigation is necessary.
295
296 Veiga et al. [26] reported higher expression of OXTR mRNA in both endometrium and myometrium
297 of late pregnant and parturient bitches than in earlier stages of pregnancy. In our study, full-thickness
298 samples were used for real-time PCR; endometrium and myometrium thus cannot be compared
299 separately. The samples of this study were run together with samples from our earlier report on non-
300 pregnant bitches [28], and the relative expression of OXTR mRNA was higher in pregnant bitches
301 than in non-pregnant ones. Expression of OXTR in the canine uterus is probably not regulated only
302 by a decrease of progesterone. In anestrous bitches with basal levels of progesterone, OXTR
303 expression does not differ from diestrous bitches with uteri under the influence of progesterone [28];
304 the expression is thus likely a part of more complex regulatory pathways. In the OD group, OXTR
305 mRNA expression was significantly decreased. In the PUI group the decrease also approached
306 significance. A large variation of OXTR mRNA expression in the CUI group may be due to the
307 heterogeneity of this group. It is also possible that in the CUI group the mechanism of dystocia is
308 different than that of the PUI and OD groups. The uterus does not contract in complete primary uterine
309 inertia, which may be due to the lack of cervical stimulus and insufficient release of OT to systemic
310 circulation. Thus, desensitization might not occur and expression of OXTR mRNA could remain
311 high. Bergström et al. [21] reported lower plasma OT concentrations in primary uterine inertia cases

than in bitches with normal parturition. In obstructive dystocia, and possibly in partial uterine inertia, uterine exhaustion possibly with paracrine or autocrine signaling may result in OXTR downregulation.

Although strict criteria were defined to include the bitches in the groups in this study, some heterogeneity probably exists. Breed diversity also increases the heterogeneity of the groups. In this study, the bitches with normal parturition were used as controls only for the blood parameters. For OXTR gene expression only ECS samples from prepartum bitches were used. Further studies are necessary to compare OXTR gene expression also with samples from bitches with normal parturition. The number of bitches was quite low (particularly in the CUI group), which may affect the results. A greater number of individuals is necessary to more properly evaluate the effect of calcium and OT treatment on OXTR. Furthermore, an uterokinetic study *in vitro* with myometrial muscle strips, as described by Gogny et al. [37], may provide information on myometrial contractions and desensitization under prolonged influence of OT. Further studies of genetic background with breeds and lines susceptible to complete primary uterine inertia are needed.

5. Conclusions

This study provides evidence of prepartum upregulation of OXTR in the canine uterus. Expression of OXTR was increased near term. A decrease in expression was observed in obstructive dystocia and may also occur in partial primary uterine inertia. However, no clear decrease in expression was observed in the CUI group, which may indicate a different etiology for inertia than in OD. The etiology in complete primary uterine inertia is more likely at the level of uterine function, such as myometrial distention beyond its capacity to contract or the lack of cervical pressure to stimulate OT

337 release. A decrease of OXTR may also occur during normal parturition; the role of desensitization
338 of OXTR in dystocia should to be clarified. It remains unclear if parturition ceases because of uterine
339 inertia or if uterine inertia occurs because of ceased parturition and desensitization of receptors.
340
341
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350
351
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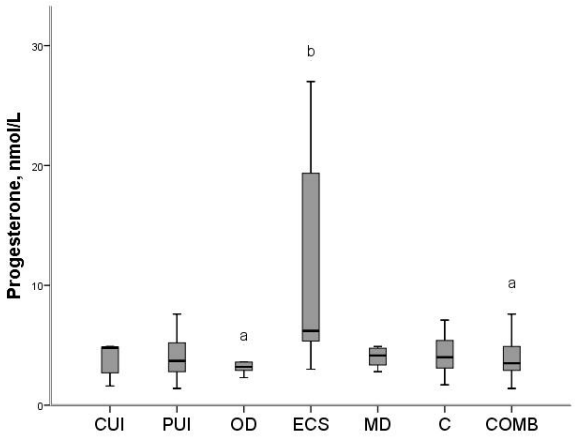
Author contributions

TMT: design of the study, RNA isolation, real-time PCR, data and statistical evaluation, manuscript writing. OV, MD, JT, TK: design of the study, data and statistical evaluation, manuscript editing. LS, BM: RNA isolation, real-time PCR, manuscript editing.

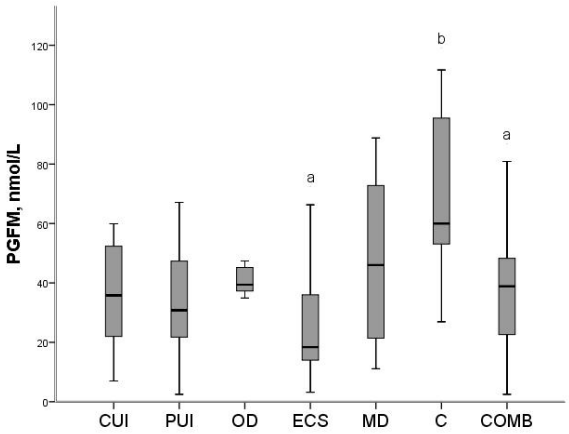
Conflicts of interest

The authors have no conflicts of interest to declare.

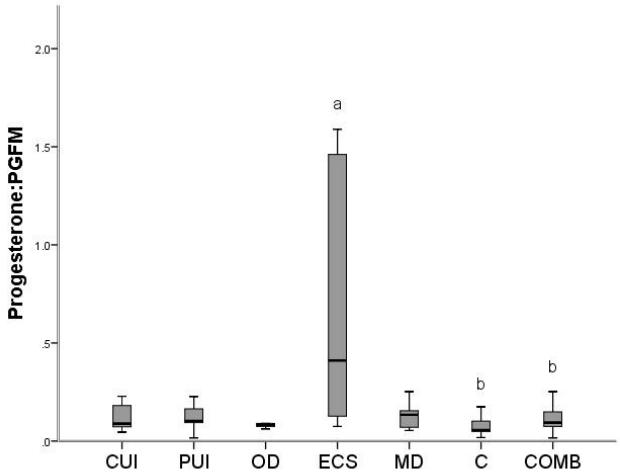
a)


Progesterone, nmol/L
30
20
10
0
b
a
a
CUI
PUI
OD
ECS
MD
C
COMB

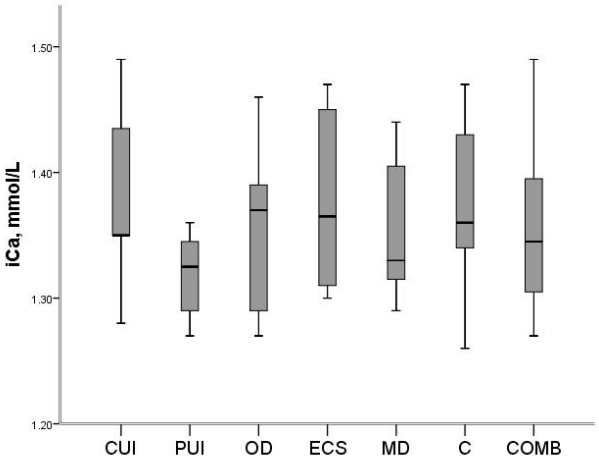
b)


PGFM, nmol/L
120
100
80
60
40
20
0
b
a
a
CUI
PUI
OD
ECS
MD
C
COMB

c)


Progesterone:PGFM
2.0
1.5
1.0
.5
.0
a
b
b
CUI
PUI
OD
ECS
MD
C
COMB

d)


iCa, mmol/L
1.50
1.40
1.30
1.20
CUI
PUI
OD
ECS
MD
C
COMB

e)

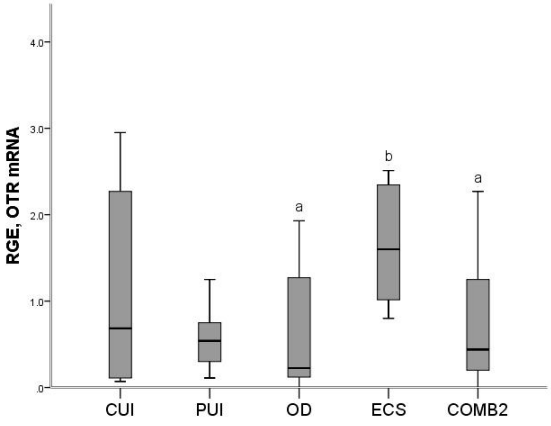

RGE, OTR mRNA
4.0
3.0
2.0
1.0
.0
a
b
a
CUI
PUI
OD
ECS
COMB2

Table 1. The individual and average data of 58 bitches divided in six groups.
Parity includes the current parturition. Medication: 0, no medication; 1, calcium and oxytocin after blood sample. n/a: not available.

Group	Mean age, min-max (years)	Mean weight, min-max (kg)	Mean litter size, min-max	Breed	Parity	Gestation length (days from the last and first mating)	Gestation length (days from ovulation)	Duration of the first stage of parturition (hours)	Duration of the second stage of parturition before intervention (hours), discharge	Litter size	Number of puppies born before dystocia/by cesarean section	Medication
1. Complete primary uterine inertia (CUI) n = 7	4.1, 2.3-6.2	23.7, 4.7-35.0	6.4, 1.0-14.0	Border Collie	1	56-61	n/a	26	3, fetal fluids	4	0/4	1
				Airedale Terrier	1	60-62	63	45	6, fetal fluids	7	0/7	0
				Karelian Bear Dog	3	68	n/a	n/a	n/a, green fluids	1	0/1	0
				Dogo Argentino	1	65	n/a	29	6, green fluids	2	0/2	0
				Chinese Crested Dog	1	62	n/a	16	4, fetal fluids	6	0/6	1
				English Springer Sp.	2	60	n/a	48	6, fetal fluids	14	0/14	1
				Labrador Retriever	1	59	60	65	6, fetal fluids	11	0/11	0
2. Partial primary uterine inertia (PUI) n = 13	5.1, 2.6-8.0	29.2, 6.5-65.0	7.0, 5.0-15.0	Mixed	1	64-65	n/a	n/a	4	5	1/4	1
				Labrador Retriever	2	60-62	62	5	4	8	5/3	1
				Dalmatian	3	63	63	6	4	6	1/5	0
				Poodle, Standard	1	61-62	62-63	7	9.5	5	3/2	0
				Labrador Retriever	2	63	n/a	10	5	8	6/2	1
				Miniature Schnauzer	1	60-61	n/a	24	3.5	7	1/6	1
				Tibetan mastiff	2	59-61	62	24	4	5	4/1	1
				Mixed	1	62	n/a	27	n/a	7	1/6	1
				Border Collie	1	57-61	n/a	22	5.5	6	4/2	1
				Bullmastiff	2	56-57	57	65	n/a, fetal fluids	15	1/14	0
				Great Dane	1	62	n/a	26	6	6	2/4	1
				Bearded Collie	1	54-56	58	14	5.5	8	4/4	1
				Giant Schnauzer	2	58	60	12	10	5	2/3	1
3. Obstructive dystocia (OD) n = 10	5.1, 2.3-8.3	23.7, 7.0-65.0	5.0, 1.0-9.0	Cairn Terrier	3	64	66	n/a	4	1	0/1	0
				Border Collie	3	59-63	63	n/a	n/a, green fluids	7	0/7	1
				Saluki	2	61	64	18	4	3	0/3	0
				Cavalier King Ch. Sp.	1	59-60	62	24	4	7	0/7	0
				French Bulldog	1	61-63	n/a	9	6	3	0/3	0
				Dog de Bordeaux	2	65-67	n/a	11	4	9	0/9	1
				Smooth Collie	3	60-61	n/a	24	8	6	3/3	1
				Cavalier King Ch. Sp.	1	56-58	59	n/a	3	4	1/3	1
				Spanish Mastiff	1	62	62	11	n/a, fetal fluids	3	0/3	1
				Border Collie	3	60-61	61	24	3	7	0/7	1
4. Elective cesarean section (ECS) n = 11	4.6, 3.1-6.6	23.9, 6.9-65.0	3.8, 1.0-10.0	Cavalier King Ch. Sp.	3	n/a	60	0	0	4	0/4	0
				Border Collie	1	64-65	64	0	0	2	0/2	0
				St. Bernard	2	n/a	61	0	0	2	0/2	0
				Cavalier King Ch. Sp.	3	58-60	60	0	0	5	0/5	0
				Great Dane	2	62-63	62-63	0	0	1	0/1	0
				Shetland Sheepdog	4	60	n/a	0	0	1	0/1	0
				Boston Terrier	2	63-63	62	0	0	2	0/2	0
				Newfoundlander	1	58-64	n/a	0	0	10	0/10	0
				Cavalier King Ch. Sp.	3	59-60	59	0	0	8	0/8	0
				Boston Terrier	1	63-65	n/a	0	0	3	0/3	0
				Golden Retriever	1	64-66	65	0	0	4	0/4	0
5. Medically treated dystocia, no cesarean section (MD) n = 8	4.7, 2.6-6.5	13.3, 4.5-20.0	5.1, 2.0-8.0	Dachshund	1	58-60	n/a	1	2	4	0/-	1
				Dachshund, miniature	1	63	n/a	15	5	6	0/-	1
				Finnish Lapphund	1	n/a	n/a	n/a	7	5	4/-	1
				Jack Russell Terrier	2	58-61	n/a	n/a	4	2	0/-	1
				Australian Kelpie	1	60	n/a	15	7	8	6/-	1
				English Springer Sp.	3	n/a	n/a	12	10	4	2/-	1
				Finnish Lapphund	2	n/a	n/a	n/a	5	6	3/-	1
				Lapponian Herder	2	n/a	63	7	5	6	2/-	1
6. Control, normal parturition (C) n = 9	4.20, 1.9-6.5	10.9, 8.0-30.0	5.4, 4.0-10.0	Cavalier King Ch. Sp.	1	n/a	60	n/a	n/a	5	-	0
				Labrador Retriever	3	n/a	60	n/a	n/a	10	-	0
				Cavalier King Ch. Sp.	4	n/a	59	n/a	n/a	4	-	0
				Pug	2	n/a	61-62	n/a	n/a	5	-	0
				Pug	1	n/a	61	n/a	n/a	5	-	0
				Cavalier King Ch. Sp.	4	61	n/a	n/a	n/a	5	-	0
				Cavalier King Ch. Sp.	1	62	n/a	n/a	n/a	4	-	0
				Cavalier King Ch. Sp.	2	n/a	58-59	n/a	n/a	5	-	0
				Cavalier King Ch. Sp.	2	n/a	60	n/a	n/a	6	-	0